PHOTOCURRENT GENERATION BY THYLAKOID MEMBRANES IMMOBILIZED IN AN ALBUMIN-GLUTARALDEHYDE CROSS-LINKED MATRIX

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SUMMARY

Thylakoid membranes immobilized in an albumin-glutaraldehyde cross-linked matrix were used for photocurrent generation by a photoelectrochemical cell in potentiostatic mode. This type of preparation was quite suited for such application because it retains a substantial volume of electrolyte within the porous network formed. This property allowed for introducing electron transfer inhibitors and artificial electron acceptors and further it permitted proper migration of electroactive species from the thylakoid membranes to the working electrode as required for efficient photocurrent generation.

INTRODUCTION

The photosynthetic membrane is able to convert absorbed solar energy into chemical energy. This property was often applied to photoelectrochemical cells of various geometry using unresolved thylakoid membranes or submembrane fractions enriched in photosystem I or in bacterial reaction center (Drachev et al., 1975; Allen and Crane, 1976; Barsky et al., 1976; Packham et al., 1980; Bhardwaj et al., 1981; Siebert et al., 1982; Sanderson et al., 1983). A major concern that is relevant to this type of study is the short active lifetime of isolated photosynthetic materials under strong illumination. A wide range of immobilization techniques have been developed recently to address the problem of the weak stability of biological functions. The better resistance of immobilized thylakoid membranes under temperature stress, long term storage or continuous illumination was correlated with a greater functional stability for photochemical hydrogen evolution, hydrogen peroxide photoproduction or photocurrent generation (Kayano et al., 1981; Ochiai et al., 1982; Cocquempot and Tomas, 1984; De la Rose et al., 1986). Immobilization in an albumin glutaraldehyde cross-linked matrix provided a better stability to thylakoid membranes than several other procedures (Cocquempot et al., 1981; Thomasset et al., 1983). In this report, we address the applicability of this type of preparation for photocurrent generation in a single compartment electrochemical cell working in potentiostatic mode.

MATERIALS AND METHODS

Thylakoid membranes were isolated from spinach leaves as previously reported (Carpentier et al., 1987) and were immobilized according to the procedure described by Thomasset et al. (1982). The immobilization mixture was poured into a petri dish and frozen at -20°C for 2h. The preparation was thawed 2 h at 4°C and samples were sliced from the resulting layer of sponge-like material.
For electrochemical measurements, we used a single compartment cell (80 μl) described elsewhere (Mimeault and Carpentier, 1988). The three electrode system (platinum working and counter electrode and a saturated calomel electrode (SCE) as the reference) was connected to a Princeton Applied Research Model 362 scanning potentiostat to establish the potentiostatic mode. The cell was filled with a sample of immobilized thylakoids in 50 mM sodium phosphate pH 7.0, 0.15 mM NaCl, 1 mM MgCl₂ with thylakoid membranes at a concentration of 0.25 mg ml⁻¹ on a chlorophyll basis. The cell was equilibrated at 23°C for 5 min before measurements. The light beam (109 W m⁻²) from a quartz halogen lamp (250 W) was directed onto the top of the cell through a fiber optic guide.

RESULTS AND DISCUSSION

Thylakoid membranes immobilized in an albumin-glutaraldehyde cross-linked matrix consists in a sponge-like material that retains a relatively significant volume of water. The electrolyte content of the matrix could be modified at will by aspiration of the proper medium under gentle vacuum. Sample were cut into the appropriate dimensions to fill the electrochemical cell chamber and the potentiostatic mode was established.

The current voltage relationship of the cell containing the immobilized membranes was monitored in the dark and under illumination. The results obtained in the dark were subtracted from that under illumination to provide the photocurrent-electrode potential behavior (Fig. 1). In the control experiment (no artificial electron acceptors added) a weak anodic photocurrent was produced which increased with electrode potential to attain a maximal value of about 1 μA at 0.8 V. However, when 10 mM potassium ferricyanide (FeCN) was added as artificial electron acceptor, a much stronger photocurrent was generated (19 μA). The acceptor thus acted as an efficient mediator to transfer electrons from the thylakoid membrane to the working electrode.

The effect of FeCN concentration is demonstrated in Fig. 2A where photocurrent induction kinetics are shown. The acceptor concentration limited the initial velocity of the induction and the maximal photocurrent produced. These inductions can be characterized by a first order reaction with a time constant not depending on FeCN concentration. In the absence of artificial electron acceptors, photocurrent was possibly generated by the membranes located in immediate contact with the platinum electrode or it was mediated by dissolved oxygen. The later is known to capture electrons from the donor side of photosystem I (Popovic et al., 1983). Nonetheless, oxygen is not as efficient acceptor as FeCN and only weak photocurrent are generated in the absence of added acceptors.

The electron transport inhibitor 3-(3,4-dichlorophenyl)-1, 1-dimethylurea was introduced in the immobilized material together with 10 mM FeCN (Fig. 2B). A strong inhibitory effect was produced under these conditions. This confirms a photosynthetic origin of the photocurrent monitored.

The immobilization in an albumin-glutaraldehyde matrix brought significant improvements over a previously reported procedure involving entrapment within a polyvinyl alcohol film (Ochiai et al., 1982). The use of the above film was in fact limited by a weak electrical conduc-